

Image Version 1.19

2 **Introduction**

Menus

3	File
8	Edit
11	Options
15	Enhance
18	Analyze
23	Video
24	Text
25	Windows

25 **Using the Tools**

Managing Windows

29	Image Windows
29	Gray Map
30	LUT
30	Histogram
31	Results

Techniques

32	Using Selections
33	Analyzing Gels
34	Redirected Sampling
35	Using the Option Key
36	Questions and Answers
37	Using Flatbed Scanners

Appendices

38	A. Frame Grabber Cards
39	B. TV Camera and Light Box
40	C. Alternate Programs
41	D. File Formats
42	E. Program Limitations
43	F. Shortcuts
44	G. New Versions and Bug Reports

Introduction

Image is a public domain program for the Macintosh II for doing digital image processing and analysis. It can acquire, enhance, measure, edit, animate, print and pseudocolor images. It reads and writes TIFF and PICT files, providing compatibility with many other Macintosh applications, including programs for scanning, processing, editing, publishing, and analyzing images. It supports many standard image processing functions, including histogram equalization, contrast enhancement, density profiling, smoothing, sharpening, edge detection, and noise reduction. Spatial convolutions, with user defined kernels up to 63x63, are supported.

Image can be used to measure lengths and x-y coordinates, and compute the average density and area of user defined regions of interest. Length and area measurements results can be calibrated to provide real world values.

Density calibration can be done against radiation or optical density standards using any user specified units. The user can select from any of eight different curve fitting methods for generating calibration curves.

It provides MacPaint-like editing of color and grayscale images, including the ability to draw lines, rectangles, ovals and text. It can flip, rotate, invert and scale selections. It supports multiple windows and 8 levels of magnification. All editing, filtering, and measurement functions operate at any level of magnification and are undoable. It uses digital halftoning to print images on PostScript printers and Floyd-Steinberg dithering for printing on non-PostScript printers.

It supports either the Data Translation QuickCapture card or Scion Image Capture 2 card for digitizing images using a TV camera. Acquired images can be shading corrected and frame averaged.

Image is written in Lightspeed Pascal from Symantec Corporation, and the source code is available. The program can be easily ported to other Pascal compilers for the Macintosh, such as MPW Pascal or TML Pascal.

System Requirements

For full operation, *Image* requires a Mac II(x, cx, or ci) with at least 2 megabytes of memory, but 4 megabytes, or more, is recommended for doing animation, for simultaneously displaying more than a handful of pictures, or for running under MultiFinder. *Image* also requires an 8-bit video card capable of displaying 256 colors or shades of gray. *Image* directly supports, or is compatible with, large monitors (but not

multiple monitors), flatbed scanners, film recorders, graphics tablets, PostScript laser printers, phototypesetters, and color printers.

File Menu

New

Creates a blank image windows which is filled with the current background color. The default size of newly created windows is 600 pixels wide by 512 pixels high. This can be changed in the More Options dialog box. The newly created window will inherit its gray map(or color palette) and any spatial or density calibration from the currently active window.

Open

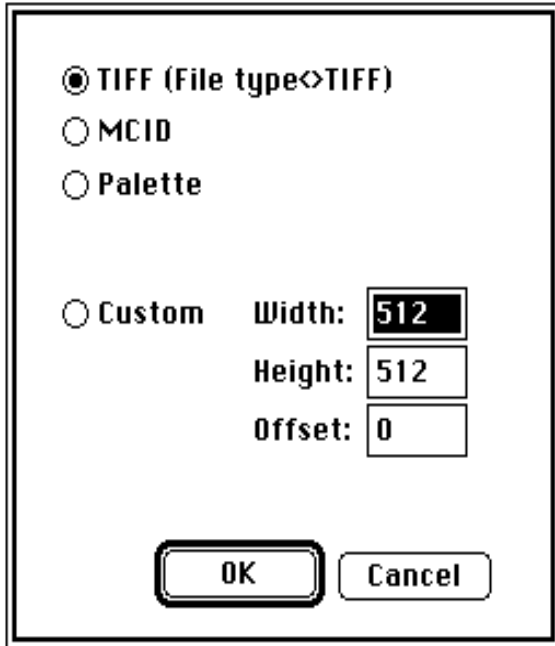
The Open command can be used to open six different file types:

- 1) 4-bit and 8-bit gray scale TIFF files, of type “TIFF”, created by many other Mac programs, including Image Studio, Digital Darkroom, and many scanner programs. TIFF files that were created on other systems, such as an IBM-PC, usually need to be opened using the Import command.
- 2) PICT files, of type “PICT”, created by many Mac programs, including MacDraw, Digital Darkroom, and PixelPaint. Hold down the Control key if you want to display the image using the current palette, instead of the one contained in the PICT file.
- 3) The *Image* picture file format(“IPIC”), which is unique to the *Image* program. These files can no longer be written by *Image*, since the IPIC format been replaced by the TIFF.
- 4) *Image* pseudocolor palettes, of type “ICOL”, which are limited to a maximum of 32 colors, and are unique to the *Image* program. Colors in pseudocolor palettes can be edited using the eyedropper tool, and the number of colors can be changed using the Set Number of Colors command.
- 5) 256 color palettes created by the PixelPaint program(type “PX05”) or by the Klutz DA(type “CLUT”). PixelPaint has a powerful palette editor which can be used to create palettes with up to 256 colors or gray values. The Klutz DA can be used to “capture” palettes used by other programs.

If you hold down the Option key(notice how Open changes to “Open All”) while selecting Open, select one image within a folder, and then click on the Open button, *Image* will open all the images in that folder, not just the one selected. This is a good way to load into memory a series of frames to be animated using the “Run Movie” command.

Import

The Import command can be used to read image files created by non-Macintosh based systems, or for importing custom color palettes. It presents a dialog box allowing you to select one of four file types. TIFF, MCID, and Custom are the three image file formats currently supported. Select Palette to import 256 color palettes.



Select TIFF to open 8-bit grayscale TIFF files imported from another system, such as an IBM PC. Select MCID to opens files created by the IBM-PC based MCID image analysis system from Imaging Research. Select Custom to open any other type of 8-bit image. If you select Custom then Width is the width of the image in pixels, Height is the number of lines in the image, and Offset specifies the number of bytes *Image* will skip before it starts reading the pixel data. For example, use an offset of 512 to skip over a 512 byte file header.

Palettes to be imported should consist of 256 8-bit red values, 256 8-bit green values, and 256 8-bit blue values, a total of 768 bytes.

Close

Closes the currently active image, Plot, or Histogram window, i.e., the one with the highlighted title bar. Hold down the Option key to close all currently open image windows.

Option-Clicking in the close box of an image window will also close all the image windows.

Save

Resaves the currently active image to the disk. In the case of a window that was newly created using New, Duplicate, or Start Digitizing, you will be prompted for a name. Files are resaved in their original format. In other words, TIFF files are resaved in TIFF format, and PICT files are resaved in PICT format. Newly created windows are saved in TIFF format.

Save As TIFF

Saves the currently active image to a file in 8-bit grayscale TIFF format. You will be asked to enter a name for the new file. Changes to Save Selection As TIFF if a rectangular selection is active, allowing you to save a subsection of the image. TIFF is the default file format in *Image*, and is usually the best format for saving digitized or scanned images. Because TIFF files are not compressed they Open and Save faster than PICT files. PICT files use a run length encoding compression scheme that is ineffective with most scanned images.

Save as PICT

Saves the currently active image to a PICT file. PICT files, which may be in color, can be exported to many other Mac programs, including PixelPaint, Digital Darkroom, SuperPaint, and MacDraw. Because they are compressed, PICT files also have the advantage of being more compact if the image contains large areas of uniform density, which is typical of computer generated images. Changes to Save Selection As PICT if a rectangular selection is active.

Warning: Don't save psuedocolored images in PICT format if you are using 32-bit QuickDraw(which is built-in to the IICI), and you plan make future density measurements, since QuickDraw may alter the pixel values in saved image.

Record Preferences

Saves(as a resource in the *Image* application file) the current state of the following *Image* program parameters. Recording preferences will not work if the Image application is residing on a write protected floppy disk or file server.

- Foreground color
- Background color
- Brush size
- Smudger diameter
- Airbrush diameter
- Color Look-Up Table mode
- Color palette position and width
- Text attributes, including font, size, style, and justification
- Extra colors
- Invert Video switch
- Measurement Options
- Profile Plot Options
- Frames averaged by Average Frames command

- Size of image created by New command
- Undo(and Clipboard) buffer size
- Width of window used for capturing with Scion frame grabber
- Apple Look Table options for images with density slicing enabled
- Frame grabber input channel
- Import command parameters

Revert to Saved

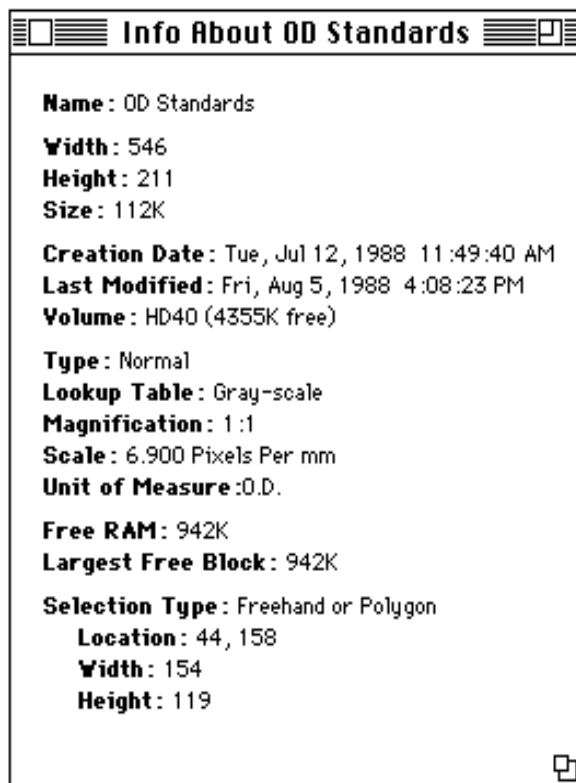
Reverts to the most recently saved version of the file, effectively undoing all changes made since the last Save command.

Duplicate

Creates a new window the size of the current rectangular selection, copies the selection to the new window, and then makes the new window the active window. The entire image is duplicated if no selection is active. The newly created window will inherit the gray map(or color palette), as well as any spatial or density calibration, of the window being duplicated.

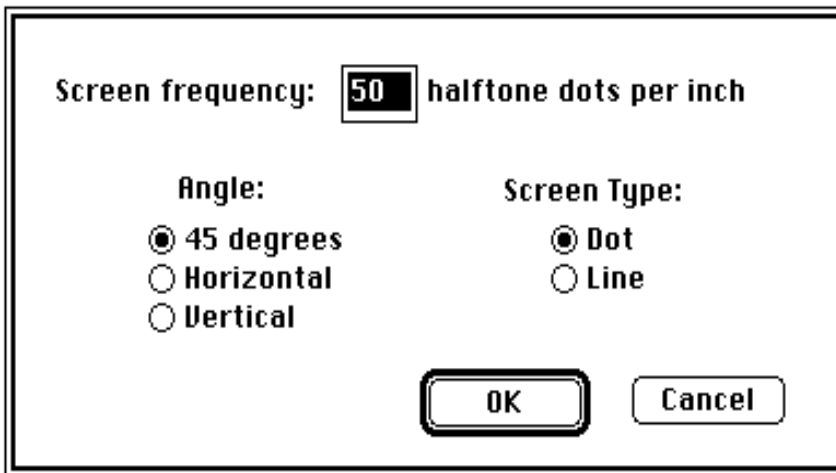
Get Info

Presents up the following window which provides information about the status of the system and of the currently active picture window.



Halftone Options

Allows you to specify how halftoning is done on the LaserWriter or other PostScript printers. Halftoning is a technique which allows grayscale images to be printed on a device whose pixels can only be black or white. This is accomplished by dividing the printer pixels into small groups (or cells) according to some pattern (called the halftone screen). Within each cell, a dot is drawn whose size is proportional to the gray value of that part of the image. A screen frequency of about 50 halftone cells per inch, providing a cell size of 6 x 6 pixels, is optimum for a 300 dots per inch laser printer.



A dialog box titled "Halftone Options" with a double border. It contains the following elements:

- Screen frequency:** A text label followed by a small square input field containing the number "50", and then the text "halftone dots per inch".
- Angle:** A text label followed by three radio button options:
 - ☒ 45 degrees
 - ☐ Horizontal
 - ☐ Vertical
- Screen Type:** A text label followed by two radio button options:
 - ☒ Dot
 - ☐ Line
- Buttons:** Two buttons at the bottom right, "OK" and "Cancel", each with a double border.

Page Setup

Lets you specify printing orientation (portrait or landscape) and scale (25% to 400%). The quality of density profile plots and calibration plots will be better if you leave the scale set at 100% and enlarge the plot window before printing the plot.

Print

This command lets you print images, plots, histograms, and the results of measurements. What gets printed is determined by which window is active at the time. The print command changes (e.g, **Print Image**, **Print Plot**, etc.) depending on which window is active.

Print Image - Prints the currently active image on PostScript printers using halftoning to simulate gray values. Any colors are converted to shades of gray (using the equivalent of the Apply Lookup Table command). If you have made a rectangular selection, only the selection will be printed.

Hold down the Option key at the start of printing to get higher quality text and line drawings on PostScript printers. In this case, printing is done using bitmap smoothing instead of halftoning. When using version 6.0(or later)of the LaserWriter driver, holding the Option key down causes the LaserWriter driver to do the halftoning and changes to “Halftone Option” have no effect. Use the new Halftone and BitMap buttons in the Print dialog box to specify what kind of printing to do.

Color printing is supported on the Tektronix 4693D, the HP PaintJet, and similar printers. On color printers and film recorders the output will be centered on the page(or slide).Use the Dither command to simulate shades of gray when printing on non-PostScript printers, such as the ImageWriter.

Print Plot - Prints the current density profile or calibration plot. The size of the printed plot is proportional to the size of the plot window.

Print Histogram - Prints the contents of the histogram window.

Print Readings - Prints the results of measurements made with the Measure Command. The Print Readings is available when you have used the Measure command at least once and the results window is active.

Print Lengths - Prints the results of length measurements made with the ruler tool. This command is available when the results window is active and you have made at least one length measurement.

Print Points - Prints the X-Y coordinate pairs resulting from using the point measurement tool. This command is available when the results window is active and you have recorded at least one point.

Save Outline As

Saves the current selection outline as a PICT file. The outline can be restored at a later time by opening the file with the Open command. The outline will be displayed within a blank image window. It can be transferred to another image window by selecting that window and using Restore selection

Save Palette

Saves the current pseudocolor palette, including its position and width, to a file.

Quit Image

Closes all image windows after asking, in each case, whether changes made during the session should be saved. Then quits to the Finder. If measurements have been made but not saved (or if a tool such as the ruler has been used), a dialog box comes up allowing a reprieve.

Edit Menu

Undo

Reverses the effect of the last editing or filtering operation. When using the Measure command, Undo deletes the most recent measurements.

Cut

Copies the contents of the current selection to the Clipboard and then fills it with the current background color.

Copy

Places a copy of the contents of the current selection on the Clipboard.

Copy will also copy the entire contents of the LUT, Plot, or Histogram window to the Clipboard if one of these is the active window. If the Plot or Histogram window is active then both the data values and the graphics will be copied to the Clipboard, allowing you to transfer plot and histogram data to other programs for plotting or statistical analysis.

You can use the eyedropper tool to copy colors from the LUT window to the Clipboard and then paste them back into another palette location. Copy and Paste of colors is limited to pseudocolor palettes, which have a maximum of 32 colors.

After using the Measure command, Copy changes to Copy Measurement, allowing you to use the Command-C keyboard shortcut to copy measurements to the clipboard.

Paste

Displays the contents of the Clipboard in the currently active image window. The pasted object is automatically selected, allowing you to move it by clicking on it and dragging. With rectangular objects, you can click and drag on the resize handle in the lower left corner to expand or contract the selection.

You can also use the paste command to paste colors copied with the eyedropper tool back into the LUT window.

If you make a rectangular selection while digitizing using the Data Translation QuickCapture(which will stop capturing), copy the selection to the Clipboard, and then paste into another window(without deleting the Camera window), then pasting will be “live” from the camera. This feature, along with the Paste Control window, allows the image currently being digitized to be aligned with a previously digitized image.

Clear

Erases the current selection using the current background color. The Delete key(or Backspace key on the small keyboard) is a handy shortcut for this command. The background color is set by clicking with the eraser tool in the LUT window. The color of the eraser indicates the current background color.

Fill

Fills the current selection with the current foreground color. You can change the color after the selection has been filled by clicking in the LUT window. The entire image window is filled if no selection is active.

The foreground color is set by clicking with the eye dropper tool in the LUT window or in the active image window. The color of the brush in the tool palette is an indicator of the current foreground color.

Invert

Inverts the current selection, or the entire image if no selection is active.

Draw Boundary

Outlines the current selection using the current foreground color and line width. Both the color and line width can be dynamically changed as long as the selection is still active.

Select All

This command is equivalent to using the rectangular selection tool to select the entire picture, including portions that may be offscreen. You can deactivate the selection by clicking on any tool other than one of the selection tools, the grabber, or the magnifying glass.

Scale Selection

Scales the current rectangular selection by a factor you enter in a dialog box. Scaling up is done using pixel replication. Use the slower Rotate and Scale command if you want independent horizontal and vertical scaling, or if you want to scale up smoothly.

Rotate Left

Rotates the contents of the current rectangular selection counter-clockwise 90°. Holding the Option key down causes the original object to be erased before being replaced by the rotated version.

Rotate Right

Rotates the contents of the current rectangular selection clockwise 90°. Hold the Option key down to erase before rotating.

Flip Horizontal

Flips the current rectangular selection horizontally around a vertical line through its center. The entire image is flipped horizontally if no selection is in effect.

Flip Vertical

Flips the current rectangular selection upside down. Flips the entire image if no selection is in effect.

Rotate and Scale

Rotates the contents of the current rectangular selection an arbitrary number of degrees. Rotation will be clockwise if you specify a positive number and counter-clockwise if you specify a negative number. You can also simultaneously scale the selection. Rotation and scaling are done using bilinear interpolation, which produces smooth looking results, unlike Scale Selection, which uses pixel replication. On the other hand, Scale Selection is much faster.

Show Clipboard

Creates a new window the size of the picture currently on the clipboard, and then displays it. This window, which will have the title Clipboard, will not be updated if the Clipboard later changes.

Options Menu

Invert Palette

Vertically flips the current color palette.

Set Number of Colors

Allows you to change the the number of colors used in the current pseudocolor palette. Any added colors will be set to shades of gray. Newly added colors can be edited by selecting the eyedropper tool and double-clicking in the LUT window on the color you want to change.

Set Extra Colors

Allows you to reserve up to six entries in the Color Look-Up Table for colors that will not be altered by routines that manipulate the look-up table. For example, you could use an extra color to add color annotation to a grayscale picture. Extra colors are displayed at the bottom of the LUT window, allowing you to edit them by double-clicking with the eyedropper Tool.

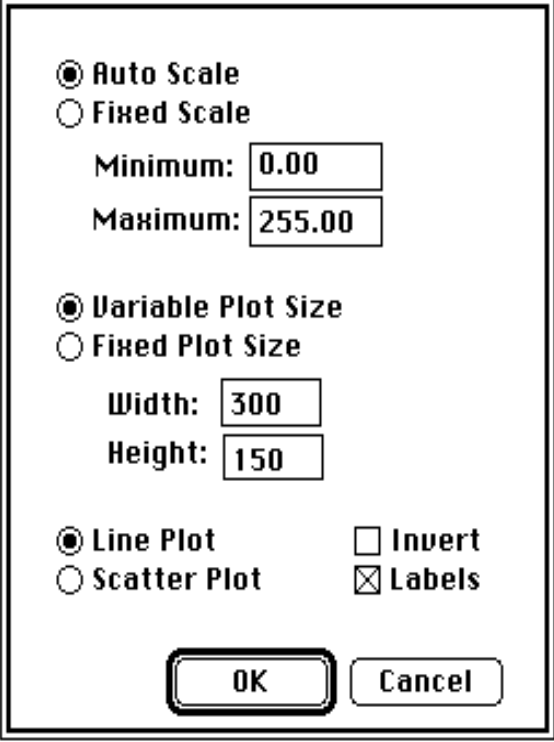
Palettes

Image currently supports four different types of look-up tables:

- 1) Grayscale** - Consists 256 shades of gray. Contrast and brightness can be altered by manipulating the transformation function displayed in the Gray Map window. This function can be applied to the pixel data in memory, thereby making any contrast and brightness changes permanent, by using the Apply Look-Up Table command.
- 2) Pseudo-color** - This is the default color mode in *Image* and was inherited from the PDP-11 version. In this mode your are limited to a maximum of 32 colors. You can use the LUT tool(the double headed arrow) to both stretch (changing contrast) and shift the colors within the LUT. Also, in this mode, you can edit colors by double clicking on them with the eyedropper tool.
- 3) System** - This is Apple's standard palette for the Mac II. It is the same as the PixelPaint's System palette. You can use the LUT tool to rotate this palette.
- 4) 256 Color Spectrum** - This is a continuous color spectrum which you can rotate using the LUT tool.

Profile Plot Options

Displays a dialog box, shown below, which allows you to set various options relating to density profile plots generated by the profile plot tool and the Column Average Plot command. In the Auto Scale mode, profile plots will be scaled depending on the minimum and maximum density values. In the Fixed Scale mode, the y-axis range is fixed and the minimum and maximum may be specified. The Fixed Plot Size mode allows specify the size of the plot window. This is useful for pasting a series of plots into an image window. Profile plot options can be made permanent using the Record Preferences command.



The dialog box is titled "Profile Plot Options" and contains several sections of controls. The first section has two radio buttons: "Auto Scale" (selected) and "Fixed Scale". Below "Fixed Scale" are two input fields: "Minimum:" with the value "0.00" and "Maximum:" with the value "255.00". The second section has two radio buttons: "Variable Plot Size" (selected) and "Fixed Plot Size". Below "Fixed Plot Size" are two input fields: "Width:" with the value "300" and "Height:" with the value "150". The third section has two radio buttons: "Line Plot" (selected) and "Scatter Plot". To the right of these are two checkboxes: "Invert" (unchecked) and "Labels" (checked). At the bottom are two buttons: "OK" and "Cancel".

☒ Auto Scale
☐ Fixed Scale

Minimum: 0.00
Maximum: 255.00

☒ Variable Plot Size
☐ Fixed Plot Size

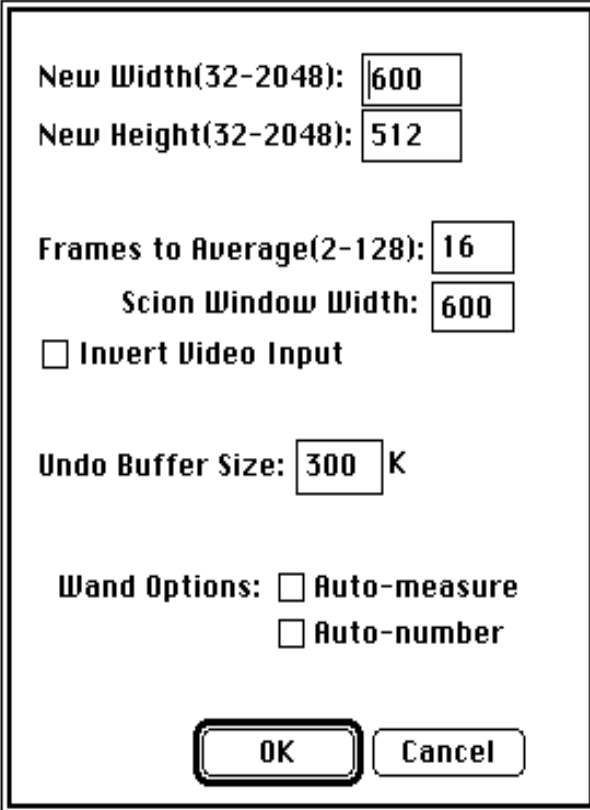
Width: 300
Height: 150

☒ Line Plot ☐ Invert
☐ Scatter Plot ☒ Labels

OK Cancel

More Options

Displays a dialog box, shown below, which allows you to change various *Image* parameters.



The dialog box contains the following controls:

- New Width(32-2048):** A text input field containing the value 600.
- New Height(32-2048):** A text input field containing the value 512.
- Frames to Average(2-128):** A text input field containing the value 16.
- Scion Window Width:** A text input field containing the value 600.
- ☐ **Invert Video Input**
- Undo Buffer Size:** A text input field containing the value 300, followed by a 'K' label.
- Wand Options:**
 - ☐ **Auto-measure**
 - ☐ **Auto-number**
- Buttons:** Two buttons at the bottom, 'OK' and 'Cancel', with 'OK' being highlighted with a 3D effect.

New Width and New Height specify the size of images created using the New command.

Frames to Average is the number of video frames that will be averaged by the Average Frames command. Scion Window Width allows you to change the width of the the window used for acquiring images when using the Scion frame grabber. This value may need to be reduced to get the Scion card to work properly with some video cards. If Invert Video Input is checked, images acquired using a grabber card will be inverted.

Undo Buffer Size allows you to change the size of the two internal image buffers(Undo and Clipboard) used by *Image*. You must Save Preferences, Quit, and then restart *Image* before any buffer size change will take effect.

When Auto-measure is checked the Measure command will be automatically called whenever an outline is created using the wand tool. If Auto-number is checked, a number,

which corresponds to the current measurement number, will be drawn in the center of outlines created using the wand tool. In addition, a white line will be drawn along the outline.

The status of all the parameters in the More Options dialog box can be made permanent using Record Preferences.

Scale to Fit Window

If checked, you can use the size box, in the lower right corner, to rescale the image in the active window. Clicking in the zoom box of an image window will switch that window to Scale to Fit mode, and then scale the window(and image) to fit the screen. You are not allowed to change the aspect ratio of an image.

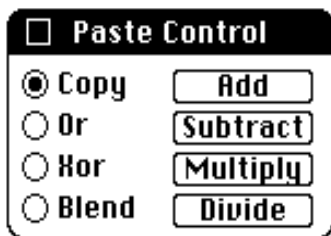
Threshold

Thresholding is used to segment an image into objects of interest and background on the bases of gray value. When thresholding is enabled objects are highlighted in red and background pixels are left unchanged. When you make area and density measurements background pixels are ignored. The gray value range used to highlight objects(called a density slice) appears in the LUT window. Use the LUT tool(the one with the double headed arrow) and click and drag near the top of the density slice in the LUT to vary its size, and click and drag near the bottom to slide it up and down. Double click on the slice with the eyedropper tool to change its color. The Measure and Show Histogram commands ignore pixels outside of the density slice when thresholding is active.

The Apply Look-Up Table command can be used to set all pixels which are within the density slice to the foreground color(or leave them unchanged) and all other pixels to the background color(or leave them unchanged). The Make Binary command will set all pixels within the density slice to black, and all other pixels to white. The wand tool can be used to outline thresholded objects.

Show Paste Control

Displays a new window, shown below, which allows you to control how image selections are pasted, or to do image arithmetic using pasted images. Notice that the Paste Control window tries to “float” above *Image's* other windows.



When Replace is selected pasting will proceed normally. When Or or Xor are selected the selection is copied to the screen using “Or” or “Xor” modes, rather than “Copy” mode, allowing you to transparently see both the object being pasted and the underlying image.

This has potential for aligning two images, and works particularly well for bi-level images, such as PICT files created by MacDraw, or images resulting from use of the Trace Edges or Dither commands. Alignment works best when the foreground color is set to black and the background to white. While in Or mode you can vary the color of the object being pasted by clicking in the LUT window. This feature allows you to overlay an image with plots of various colors. In Blend the resulting image is a blend(average) of the source and destination. Blend mode is too slow for doing image alignment, except for very small selections.

Or mode can be used to color objects in binary(black and white) images created by *Image* or programs such as by SuperPaint and MacDraw. Simply select the object you want to color, Copy, switch to Or mode, then select a color by clicking in the LUT window.

Clicking on Add, Subtract, Multiply, or Divide causes the specified image arithmetic operation to be performed and terminates the paste operation. For example, clicking Subtract will subtract the selection being pasted from the current window. These operations are done in two passes. In the first pass, *Image* determines the minimum and maximum gray values that would result from performing the operation. In the second pass, the image arithmetic is actually performed, and the resulting pixel values are scaled to the range of 0 to 255.

Subtraction is probably the most useful arithmetic operation. It can be used to subtract the background(the image with features of interest removed from view) from an image. It can also be used to detect differences between two images or for motion analysis.

The arrow keys can be used to nudge the selection currently being pasted in any of four direction by a single pixel. You can get interesting effects, including edge detection, by using the arrow keys to offset an image by one pixel, and then subtracting it from itself. To see how this works, try Select All(Command-A), Copy(Command-C), Paste(Command-V), Down Arrow, Right Arrow, and Subtract.

Enhance Menu

Filtering Functions(Smooth, Sharpen, etc.)

These functions, with the exception of Reduce Noise and Dither, are implemented using 3 x 3 spatial convolutions, where the value of each pixel in the selection is replaced with the weighted average of its 3 x 3 neighborhood. For correct operation, they require a

grayscale image, or a pseudocolored image that started out as a grayscale image. Filtering is not limited to rectangular selections. The entire image will be filtered if no selection is active. Filtering operations can be aborted by typing Command-Period.

The 3 x 3 tables shown below are the coefficients for the filters which use spatial convolution. The popularity of plus and minus one in these tables is due to the fact that multiplication by one is very efficient, i.e, it is unnecessary.

Smooth - This filter blurs(softens) the selection area. It can be used to reduce noise in an image. Hold the Option key down for increased blurring.

1	1	1	1	1	1
1	4	1	1	1	1
1	1	1	1	1	1

(If Option key held down)

Sharpen - Increases contrast and accentuates detail in the selection, but may also accentuate noise. To minimize this problem, you can Smooth and/or Reduce Noise before using Sharpen. Hold the Option key down for increased sharpening.

-1	-1	-1	-1	-1	-1
-1	-1	-1	-1	-1	-1
-1	-1	-1	-1	-1	-1

(If Option key held down)

Trace Edges - Produces a binary image with a black background and white outlines representing objects in the original image. Two convolutions are done, generating vertical and horizontal derivatives and the larger of the two result is used. If the resulting pixel is above a threshold value it is set to white, otherwise it is set to black. You can increase or decrease the number of edges by preprocessing the image. Increasing contrast and Sharpening will increase the number of edges found, decreasing contrast and smoothing do the opposite. If you hold down the Option key the thresholding step will not be done, allowing you to do it yourself.

1	1	1	-1	0	1
0	0	0	-1	0	1
-1	-1	-1	-1	0	1

Reduce Noise - This is a “median filter”, where each pixel is replaced with the median value of its 3 x 3 neighborhood. This a time consuming operation because, for each pixel in the selection, the nine pixels in the 3x3 neighborhood must be sorted and the center pixel replaced with the median value(the fifth). For a demonstration of the effectiveness of median filtering try removing random spot noise generated using an air brush with a diameter of around 50.

Dither - Uses the Floyd-Steinberg error diffusion algorithm to convert the current selection to a binary(black and white only) image. Dithering can be useful for exporting pictures to applications such as MacPaint, MicroSoft Word or MacDraw that can only accept binary images. Dithering can also be used for printing on the ImageWriter, or other non-PostScript printers. The quality of dithered images can frequently be improved by increasing contrast using the Gray Map window before doing the dithering. Color images should be converted to grayscale using the Apply Lookup Table command before being dithered.

Convolve - Does spacial convolutions using kernels, which are read from a text file, than can be up to 63 x 63 in size. Desk accessory text editors, such as MockWrite or MiniWriter, are particularly convenient for creating or examining these kernels.

For an example, use a text editor to create the following file and then use the Convolve command to try it out.

```
0  0  0 -1 -1 -1  0  0  0
0 -1 -1 -3 -3 -3 -1 -1  0
0 -1 -3 -3 -1 -3 -3 -1  0
-1 -3 -3  6 13  6 -3 -3 -1
-1 -3 -1 13 24 13 -1 -3 -1
-1 -3 -3  6 13  6 -3 -3 -1
0 -1 -3 -3 -1 -3 -3 -1  0
0 -1 -1 -3 -3 -3 -1 -1  0
0  0  0 -1 -1 -1  0  0  0
```

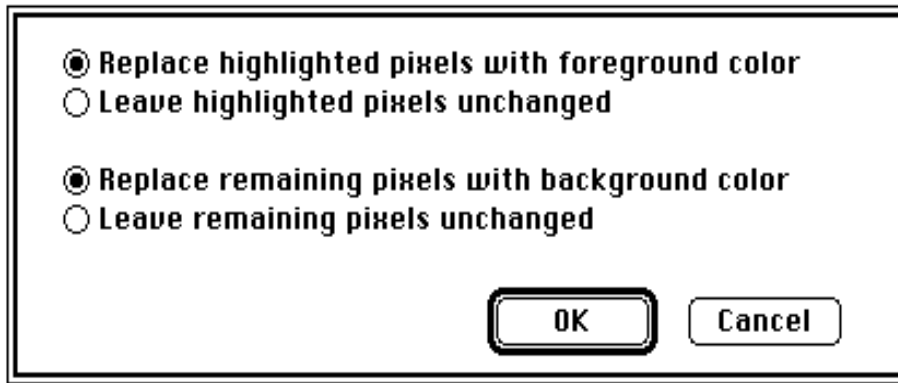
This is a 9 x 9 “Mexican hat” filter which will do both smoothing and edge detection in one operation. Each line should be terminated with a carriage return, and the coefficients should be separated by one or more spaces, or by a tab.

Equalize

Performs histogram equalization based on the density histogram of the current selection. A new gray map look-up function is generated which more evenly distribute the gray values of the image. This will usually improve contrast. The newly created look-up table function is displayed in the Gray Map window. Use the Apply Look-Up Table command to make the contrast change permanent. Equalization only works with grayscale images.

Apply LUT

Applies the current look-up table to each pixel in the current selection (or of the entire image if there is no current selection) and then restores the default look-up table(the identity function). This modifies the gray values so that when the image is viewed using the default look-up table it will look the same as it did before. This command provides a way of making brightness and contrast changes permanent. It can be used to posterize(reduce the number of gray values) an image by loading and Applying, for example, a palette consisting of four shades of gray. It can also be used to convert color images to grayscale, and to convert a thresholded image to binary(black and white only).



If thresholding is in effect the above dialog box will be displayed, allowing you to process pixels highlighted by the thresholding in one way, and all other pixels another way.

Binary

This submenu is used to convert grayscale images to binary(black and white only) and also to process these binary images.

Make Binary - Converts the current image to binary format by setting pixels highlighted by thresholding to black(255), and all other pixels to white(0). Thresholding must be in effect in order to use this command. There are two ways to enable thresholding: by using the Threshold command, or by clicking on the threshold button in the Gray Map window.

Erosion - Removes pixels from the edges of objects in a binary images, where contiguous black areas in the image are considered objects, and the background is assumed to be white. A pixel is removed(set to white) if four or more of its eight neighbors are white. Erosion separates objects that are touching and removes isolated pixels.

Dilation - Adds pixels to the edges of objects in a binary images. A pixel is added(set to black) if four or more of its eight neighbors are black. Dilation connects discontinuous objects and fills in holes.

Opening - Performs an erosion operation, followed by dilation, which will smooth objects and remove isolated pixels.

Closing - Performs an dilation operation, followed by erosion, which will smooth objects and fill in small holes.

Outline - Generates a one pixel wide outline of objects in a binary image.

Skeletonize - Repeatably removes pixels from the edges of objects in a binary image until they are reduced to single pixel wide skeletons. Command-period can be used to abort the thinning process.

Change Color

Changes the color of all pixels in the selection(or the entire image if no selection is active) that are in the current foreground color to the current background color. For example, to change a solid white background to black you would set the foreground color to white, the background color to black and then use Change Color.

To be more precise, Change Color changes all pixels using the current foreground index value(a number in the range 0 to 255) to the current background color index value. It is possible that a given color on the screen could be represented by several different index values. In this case, Change Color may not behave as expected.

Analyze Menu

Measure

Computes the mean, standard deviation, minimum and maximum intensity of the pixels in the current selection. In addition, you can use the Measurement Options command to enable other measurements, such as perimeter length. Undo will delete the last measurement. If the minimum density is less than 1, or the maximum is greater than 254, then a message will be displayed indicating the possibility of saturation. If thresholding is enabled only thresholded pixels will be included in the computation.

If Set Scale has been used to establish the spatial scale, then the area of the selection will be given in calibrated units, such as square millimeters. If Calibrate has been used to perform density calibration, then density readings will be converted, using the standard curve generated by Calibrate, and the results reported in calibrated units, such as optical density or isotope concentration.

Show Results

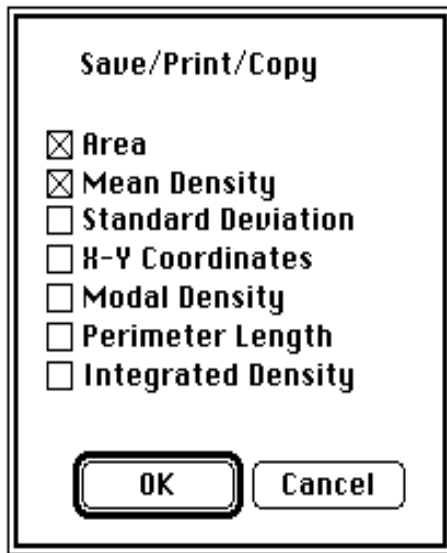
Displays a list of the current measurements. At the bottom of the window are buttons that can be used to Export, Print, or Copy(to the Clipboard) the readings, or to Reset the measurement counter. Show Results displays length measurements if the current tool is the ruler and you have made at least one length measurement. Likewise, it displays X-Y coordinates if the current tool is the pointing tool, and you have recorded X-Y coordinates.

Area Measurements					
	Area	Mean	S.D.	X	Y
1.	7425	0.344	0.100	47.817	16.761
2.	5670	0.649	0.065	29.859	14.648
3.	2700	1.060	0.030	7.606	13.380
4.	1947	0.685	0.060	28.662	22.746
<div>Export Print Copy Reset</div>					

Export saves the measurements as a tab delimited text file that can be Opened by many Mac programs, including Excel, StatView, and Cricket Graph. For correct display, it may be necessary to adjust tab settings when opening an exported file with a word processing program, such as MacWrite.

Measurement Options

Allows you to specify which measurement will be displayed(and Exported, Printed or Copied) by Show Results. Double-clicking on the polygon tool will also bring up this dialog box.



Area - Area of the selection in square pixels. Area will be given in calibrated units, such as square millimeters, if you have used Set Scale to do spatial calibration.

Mean Density - Mean density is given in calibrated units if you have used the Calibrate command to generate a standard curve.

Standard Deviation - This is the standard deviation of the pixel densities used to generate the mean density.

X-Y Coordinates - Center of the selection's bounding rectangle.

Modal Density - Most frequently occurring density level within the selection. Corresponds to the highest point in the density histogram.

Perimeter Length - Length around the outside of the selection. Only computed for freehand and polygon selections.

Integrated Density - Used to measure the size(volume) of spots(such as protein spots on a two-dimensional electrophoresis gel). Computed using the following formula:

$$\text{IntegratedDensity} = N * \text{MeanDensity} - N * \text{BackgroundDensity}$$

where N is number of pixels in the selection, and BackgroundLevel is the modal density after smoothing the density histogram. If there isn't a well defined peak in the histogram then the background level may be computed incorrectly, resulting in a negative integrated density.

Show Histogram

Displays a plot showing the distribution of gray values within the selection. The plot shows, for each of the possible 256 gray values, the number of pixels in the selection that have that gray value. Histograms are not limited to rectangular areas. When the cursor is over the Histogram window, X(the gray value) and Y(the number of pixel with that gray value) are displayed dynamically in the Results Window.

Using the Copy command when the histogram window is the active window will copy both the histogram plot(as a PICT) and the histogram data values(as a single column of text) to the clipboard.

Column Average Plot

Generates a density profile plot, where the width of the plot is equal to the width of the current rectangular selection and each point in the plot represents the average intensity of the pixels in the corresponding column in the selection. The Profile Plot Options dialog box can be used to control various aspects of these plots.

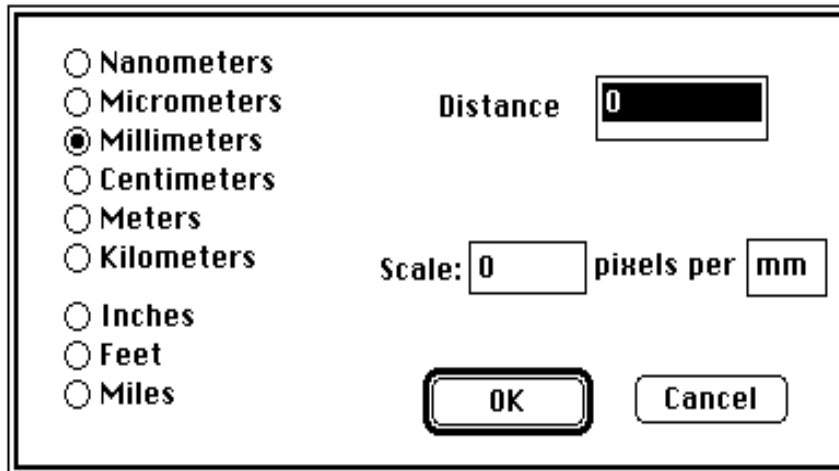
Using the Copy command when the Plot window is the active window will copy both the column average plot(as a PICT) and the data values(as a single column of text) to the clipboard.

3D Plot

Generates a 3-dimensional display of the current selection. If no selection is active the entire image will be plotted. The 3-D plot will replace the current image. You can use Command-Period to abort plotting and Undo(Command-Z) to restore the image. You can exaggerate the vertical scale in 3D plots by increasing the contrast of the image before plotting using the controls in the Gray Map window.

Set Scale

Performs spacial calibration so that results from length and area measurements can be presented in calibrated units, such as millimeters. Before using this command you must first measure a known distance along a ruler or other scale using the ruler tool. Then, simply enter this value in the Set Scale dialog box. Double-clicking on the ruler will also present this dialog box.



The dialog box is titled "Set Scale" and contains the following elements:

- A vertical list of units on the left, each preceded by a radio button:
 - ☐ Nanometers
 - ☐ Micrometers
 - ☒ Millimeters
 - ☐ Centimeters
 - ☐ Meters
 - ☐ Kilometers
 - ☐ Inches
 - ☐ Feet
 - ☐ Miles
- A "Distance" label followed by a text input field containing the value "0".
- A "Scale:" label followed by a text input field containing the value "0", the text "pixels per", and another text input field containing the value "mm".
- Two buttons at the bottom: "OK" and "Cancel".

Calibrate

Allows you to calibrate to a set of density standards, such as a calibrated optical density step tablet, or radioactive isotope concentrations. Before using this command, use the Reset command to set the measurement counter to zero, and use the Measure command to record the mean density of each of the standards. To save time, you can click on the Open button to read in the actual(as opposed to measured) standard values from a text file.

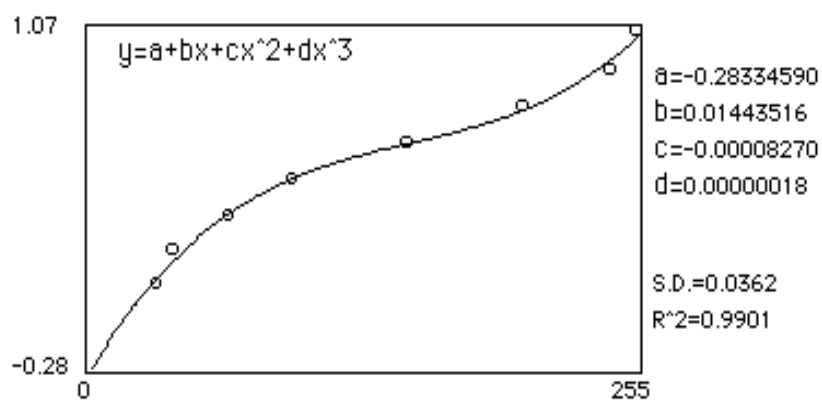
If you hold down the option key when invoking Calibrate you will be allowed to change the measured values. This feature allows you to enter measurements acquired previously or acquired from another system.

1	30.80	.06
2	38.34	.20
3	64.25	.34
4	94.26	.49
5	147.67	.64
6	202.18	.79
7	243.07	.94
8	255.50	1.1
9		
10		
11		
12		
13		

☐ Straight Line
☐ 2nd Degree Polynomial
☒ 3rd Degree Polynomial
☐ 4th Degree Polynomial
☐ 5th Degree Polynomial
☐ Exponential
☐ Power
☐ Log

Unit of Measurement:

Calibration Dialog Box



Resulting Calibration Curve

Reset

Reset the counters used for area(density), length, and X-Y coordinate measurements to zero.

Restore Selection

Returns the selection outline to its original position immediately after an operation that has removed it. If there is an outline currently active, this command restores the previous one. Can also be used to transfer a selection outline from one window to another, or for redirected sampling(see below).

Number Selection

Draws the current measurement count at the center of the current selection and outlines the selection in white. Can be used to keep track of which objects have been measured when doing redirected sampling(see Techniques).

Video Menu

Start Digitizing

Captures TV camera images using either the Data Translation DT2255-60Hz QuickCapture card or the Scion Image Capture 2. While digitizing using the DT2255, you are allowed to change contrast, alter color schemes, zoom, or pan. Clicking in the Camera window with any tool except the magnifying glass or the grabber will cause digitizing to stop. While digitizing is in progress Start Digitizing changes to Stop Digitizing. Histograms will be continuously computed and displayed if you use the Histogram command after you have started digitizing with the Data Translation QuickCapture card. Hold down the option key to digitize using the whole screen with the Scion card. Newly created Camera windows will inherit the gray map(or color palette), as well as any spatial or density calibration, of the currently active window, if there is one.

Average Frames

Averages two or more video frames to reduce time-varying random noise. Averaging will be done only for the current rectangular selection, or for the entire image if there is no selection. The number of frames to be averaged is specified using the More Options command.

Hold down the option key to do frame summation, rather than frame averaging. Frame summing increases image quality and contrast in low light situations. Pixel values resulting from frame summing are scaled to a range of 0 to 255.

Save Blank Field

Use this command to save a median brightness blank field which will be used to correct for nonuniform illumination and for the nonuniform response of the video camera. Before using this command you must start digitizing and adjust the lens diaphragm and/or lightbox intensity so that the average pixel value, as shown by the dynamic pixel value displayed in the Results window, is approximately 128.

The Stop Digitizing command will use the reference image previously acquired by Save Blank Field to correct shading errors in the newly acquired image. It does this by dividing each pixel in the newly acquired image by a correction factor computed for each of the 300,000 pixels in the blank field. The correction factor is generated by dividing each blank field pixel value by the mean blank field density. You can digitize a single frame without shading correction by holding down the Option key while selecting Stop Digitizing. Close the “Blank Field” window if you want to stop doing shading correction.

Select Channel

Allows you to specify which input channel is used by the QuickCapture or Scion frame grabber board.

Make Movie

Captures a series of video frames the size of the current selection. If no selection is in effect, the frames are the same size as the camera window. *Image* will attempt to open the number of windows required to hold the number of frames you specify. You will be asked to enter the time to delay between frames. Entering zero will result in the fastest possible sampling rate. Full 640x480 frames can be acquired at a rate of about six per second. Rates of up to about 20 frames per second are possible with small selections. You are allowed to enter delays of less than one second. For example, enter 0.5 to sample at 2 frames per second. The number of the current frame is shown in the Results window. Type command-period to stop acquiring frames. A useful movie making shortcut is the one where you option-click in the close box of an image window to close all of the currently open windows.

Run Movie

Animates the currently opened images by repeatedly drawing them to the screen at the location of the currently active window. If the images are not all the same size, or if you

hold the Option key down, the screen will be erased to the current background before the animation starts. The images will be drawn in the order in which they are listed in the Windows menu. The speed of drawing can be controlled by pressing keys '1' through '9'. The right and left arrow keys can be used to single step through the images. Press the mouse button to stop the animation.

Use the Open All command(select Open with the Option key down) to load a series of images which make up a movie. You can also use the Finder's Open command to cause *Image* to load a series of images. *Image* will close all open image windows if you hold the option key down in click in the close box of any image window.

Photo Mode

Erases the entire screen to the current background color and then redraws the current image window without the menu bar, title bar, tool palette, etc. You can change the background color by selecting the eraser tool and clicking in the LUT window. Holding down the Option key while selection this command will cause the image to be drawn starting at the top of the screen using the space formally occupied by the Menu Bar.

Text Menu

Text handling in *Image* is similar to that in Macintosh painting programs such as MacPaint, SuperPaint, and PixelPaint, with the exception that you currently can only change characteristics, such as size and style, of the last line entered. You are allowed to change the color(by clicking in the LUT window), size, typeface, style and justification of the current line of text after the text has been entered. The background color can be changed by option-clicking in the LUT window.

Windows Menu

Next Window

Deactivates the current image window and activates the next window in the list of window shown in the Windows Menu.

Stack Windows

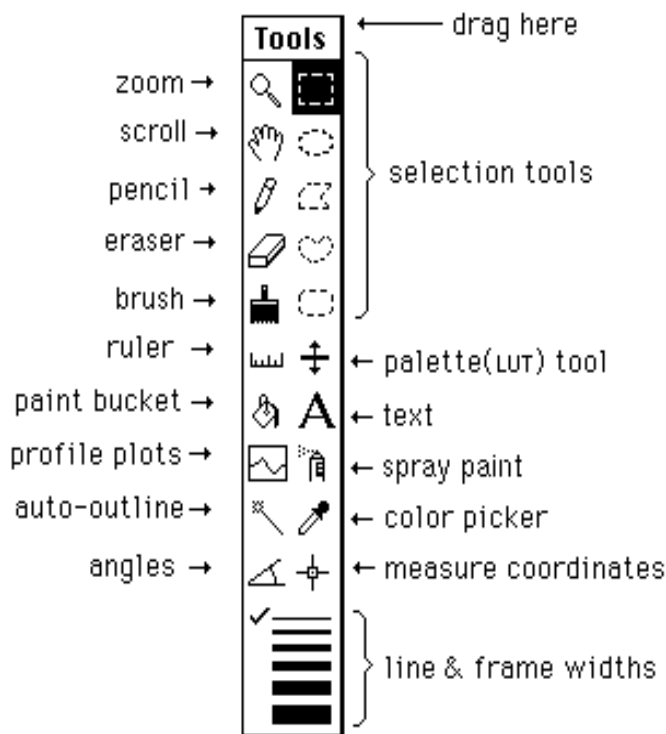
Expands all the image windows to their original size and redraws them slightly offset from each other. If you hold down the Option key all the windows will be drawn in the same location, at the upper left corner of the screen, next to the tool palette.

Tile Windows

Contracts all the images windows and repositions them so that they will fit on the screen without overlapping. If you hold the Option key down the tiled windows will be drawn using “Scale to Fit” mode. Click on the Zoom Box of a tiled window to expand it to fill the screen.

Using the Tools

The window containing the tool palette, as well as all the other windows displayed by *Image*(with the exception of the Scion Camera window), can be freely moved around the screen. All the tools work at any of the eight available levels of magnification.





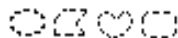
Magnifying Glass - Click within the active image window to zoom. Double-click on the magnifying glass to unzoom. Option-click(or use Undo) within the image window to unzoom in steps. Notice how the plus sign changes to a minus sign when you hold the option key down.



Scrolling Tool(Grabber) - Moves images within windows. When using other tools, with the exception of the text tool, you can temporarily switch to the grabber by holding down the space bar.



Selection Rectangle - Use this tool to specify a rectangular subregion for use by commands in the Edit, Functions and Analysis menus. Rectangular selections can be Saved, Copied, Cleared, Filled, Duplicated, Scaled, Flipped, Rotated, Inverted, filtered, or Measured. Hold down the Shift key to constrain the selection to be square. Double-click to select the entire image. As the rectangle is being drawn, its width and height are shown in the Results window.



Oval, Polygon, Freehand, Rounded Rectangle - These are outlining tools for defining non-rectangular subregions for use by various commands in the Edit, Functions and Analysis menus. Regions defined by these tools, along with the rectangle tool, can Copied, Filled, Cleared, Inverted, Framed, filtered, or Measured. The Fill command allows you to change colors after a region has been filled by clicking in the LUT window. Similarly, Draw Boundary allows you to change the line width by clicking on the lines in the Tools window. Double-click on the polygon tool to bring up the Measurement Options dialog box. Use the arrow keys to “nudge” selections one pixel at a time.



Pencil - Draws thin lines using the current foreground color. Option-click to pick up the color under the pencil. This works in either the current image window or the LUT window. It is not necessary to hold down the Option key to pick up colors from the LUT window. Holding down the Shift key causes pencil movements to be constrained to be either horizontal or vertical.



Eraser - Erases to the current background color. Option-click to pick up background colors from within the image window. The eraser can also pick up background colors

from the LUT window. Holding down the Shift key causes eraser movements to be constrained to be either horizontal or vertical. The color of the eraser indicates the current background color. The background color is used by the Cut and Clear commands and as the background color for text. Double-click on the eraser to erase the active image window.



Paint Brush - Draws in the current foreground color. Option-click to pick up the color under the brush. It is not necessary to hold down the Option key to pick up colors from the LUT window. The color of the brush indicates the current foreground color. Double-click on the brush to change its size. Holding down the Shift key causes brush movements to be constrained to be either horizontal or vertical.



LUT Tool - This tool is used to dynamically modify the Color Look-Up Table(LUT) by clicking and dragging in the LUT window. It is also used to manipulate the density slice when thresholding is enabled. Double-click on this tool to enable/disable thresholding.



Airbrush Tool - Double-click to change the brush diameter.



Ruler

Draws straight lines and measures linear distances. To measure a distance(or draw a line), click at the starting point, drag to to the ending point, and then let the mouse button up. The horizontal distance(X), vertical distance(Y), and total distance will be dynamically shown in the Results window as the line is drawn. If you are measuring, and don't want to leave the line as a reference, use the Undo(Command-Z) to erase it.

Hold down the Option key to measure distances along an irregular path. Tracing in this case is done using a technique similar to how the polygon outlining tool works. Terminate tracing by double clicking. It is only necessary to hold the Option key down at the start of tracing.

Lines can be constrained to be either vertical or horizontal by holding down the Shift key. Line width is specified by clicking on the lines at the bottom of the Tools window. Distances are given in pixels unless Set Scale has been used to perform spatial calibration. Use Show Results to display length measurements. Double-click on the ruler to bring up the Set Scale dialog box.



Automatic Outlining Tool(Wand)

Traces the edge of a thresholded object and, optionally, measures and/or numbers it, producing a standard *Image* outline in the process. To use this tool, you must be currently thresholding, or working with a binary image created with the Make Binary command. There are two ways to do thresholding. The first method is to use the Threshold command(or double click on the Wand tool) to enable thresholding, and then use the Wand tool to manipulate the resulting density slice. The second method is to click on the threshold button in the lower right corner of the Gray Map window, and then click and drag the vertical line to control the threshold.

The object to outlined should first be thresholded. Next, click inside the object near the right edge. Imagine a turtle that starts moving right from where you click looking for an edge. Once it finds an edge, it will trace it in a counter clock-wise direction until it returns to the point where it first found the edge.

You can specify that the object be automatically measured and/or numbered by using the More Options command. The numbers correspond to measurement numbers. If you are numbering the objects, but not measuring them, the numbers will not automatically be incremented. Undo can be used to remove the most recent number, along with the corresponding measurement, if any.



Density Profile Tool

Displays a plot of the gray values along an arbitrary line. You generate this line in the same way you use the ruler tool to draw lines or to measure lengths. Hold down the option key if you want the line drawn for reference purposes. Averaging will be performed if the line width is greater than one. For example, assume the maximum line width is selected by clicking on the thickest line at the bottom of the tool palette, then each data point plotted is the average of eight pixels. The Plot window, unlike the Histogram window, may be resized. Lines can be constrained to horizontal or vertical by holding down the Shift key.

Plots can be copied to the Clipboard and then pasted into a picture window. In addition to the plot, the Copy command also copies the plot data to the Clipboard as a single column of numbers, where it can be pasted into analysis and plotting programs, such as KaleidaGraph.

Various aspects of the plots produced by this tool can be altered using the Profile Plot Options command. Double-click on this tool to bring up the Profile Plot Option dialog box.



Paint Bucket

This is a MacPaint-like Paint Bucket. It causes all pixels located where paint can leak from the starting point (the end of the paint coming from the bucket) to be changed to the foreground color. In conjunction with density slicing, it can be used for measuring areas under profile plots. Profile plots must first be pasted into an image window before they can be filled using the Paint Bucket.

There is currently a bug which prevents paint from leaking near the right edge of images with widths that are not evenly divisible by eight.



Text Tool

When only words will do. Allows typing in the Font and Style chosen in the appropriate menus. Various attributes of the text, such as font, size and color, can be changed after the text has been entered, but once another tool has been chosen, or you have typed Return, the text becomes part of the image's bitmap.

Hold down the option key and the text tool will automatically type for you the most recent area measurement. If you prefer, it will enter the most recent mean density reading if you use Measurement Options to disable area measurements. Repeated option-clicking will enter previous readings, starting with the most recent one. Using Show Results will allow you to automatically type a set of readings a second time.



Eyedropper - Picks up colors from the active picture window and from the LUT window. Option-click to pick up background colors. If you are using pseudocolor, double-clicking on a color in the LUT window causes the Color Picker dialog box to be displayed, allowing you to modify that color. Double-clicking in the LUT window also allows you to change the density slice color when *Image* is in the density slicing mode.



Angle Tool - Measures the angle formed by two lines drawn through a point by this tool. The value is shown interactively in the results window. Undo can be used to delete the lines if they are not wanted.

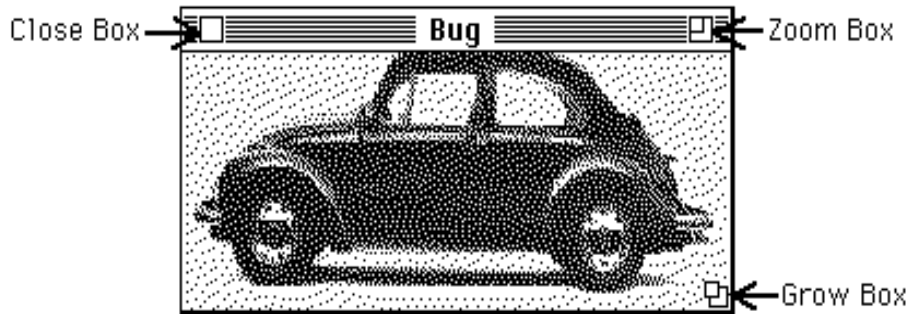


Counting Tool - Counts objects and records their X-Y coordinates, leaving markers so that objects are not counted twice. Markers are drawn in the current foreground color and their size is related to the current line width. Use the Show Results command to display the X-Y coordinates.



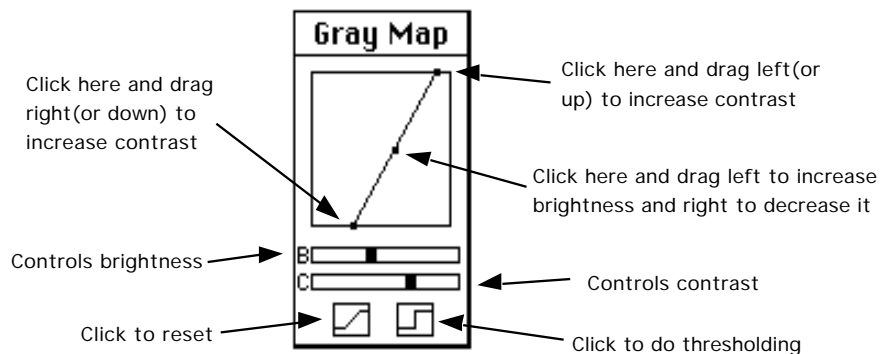
Line Width - Allows user to choose the line width used by the ruler and profile plot tools and by the Draw Boundary command. The lines are 1, 2, 3, 4, 6, or 8 pixels in width.

Image Windows



Images are displayed within image windows, such as the one above, which are created using New, Open, Import, or Duplicate. The size of image windows created using the New command can be specified in the More Options dialog box. The Close Box closes the window and frees the memory used to store the image. Hold down the option key when clicking in the close box to close all image windows. The Zoom Box switches the window to “Scale to Fit Window” mode and resizes the window to make it as large as possible, while still maintaining the same aspect ratio. You can restore the window to its original size by clicking again in the Zoom Box, or by using Unzoom. Note that the magnifying Glass and the Grabber tools do not work in Scale to Fit mode. The Grow Box is used for resizing the window. If you are in the Scale to Fit mode the image will be rescaled to fit any resized window. You can use the Grow Box to make the window larger than its original size if you have zoomed in using the magnifying glass.

Gray Map Window



This window is used to vary brightness and contrast by loading different functions into the video look-up table. To vary brightness, click and drag inside the frame, which moves the plot(a piece-wise linear density transformation function) horizontally. This changes the Y-intercept of the function while maintaining the slope fixed. To change contrast, click and drag in the margin to move the two points which define the function. A good strategy for improving the contrast of grayscale image is to click(outside the frame) in the lower left hand corner, then drag horizontally to the right until the image starts to saturate. Similarly, click in the upper right hand corner, and drag horizontally to the left. If you are using a color palette, clicking in this window switches to grayscale mode. Double-click to reset the look-up table to a 256 gray value ramp(an identity function).

LUT Window

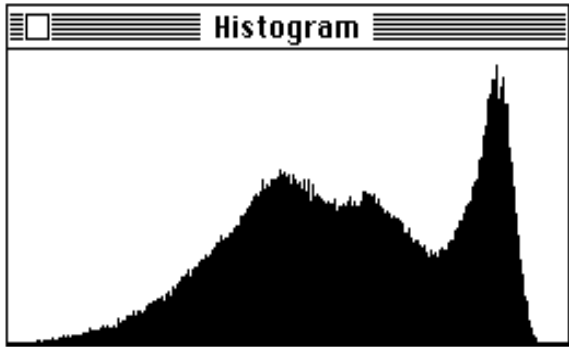


This window displays the contents of the video look-up table(LUT), which is used to transform each of the 256 possible pixels values into one of 16 million possible screen colors. Several types of look-up tables(also called palettes) are possible, including grayscale, pseudocolor, and custom.

Click in the LUT window with any of the drawing tools to pick up a new drawing (foreground) color. 256 color palettes, such as the one produced by the 256 Color Spectrum command can be “rotated”, but not stretched. Click in the LUT widow with the eraser, or Option-click with any other tool, to pick up a new background color. Use Save to save the modified palette with the image, or Save Palette As to save the palette separately.

The default pseudocolor palette consists of 20 colors. Click and drag near the bottom of the palette to move it within the LUT. Click and drag near the top of the palette to stretch it. Double-click on a color with the eyedropper tool to edit it.

Histogram Window



This window displays the gray value histogram produced by the Show Histogram command. This is a function that shows, for each of the 256 possible gray values, the number of pixels within the selection that have that gray value. The actual histogram values will be dynamically displayed in the Results window whenever the cursor is over the histogram window. Both the histogram plot and the 256 data values can be copied to the clipboard(using Copy) whenever the histogram window is active.

Results Window

X, Y and Value at the top of the Results window are dynamically updated variables that have different meanings depending on which window the cursor is in.

Image Window - X and Y are the current cursor coordinates, where the origin of the coordinate system is at the lower left-hand corner and positive X is to the right and positive Y is up. Value is the grayscale value of the pixel at the current X-Y coordinates. The range of values is from 0(white) to 255(black). In the case of color or pseudocolor images, Value is the index used by the color look-up table. If you are printing an image, X is the line currently being printed and Y is the total number of lines.

LUT Window - Value is the grayscale value(look-up table index) pointed to by the eyedropper. You can use this information to set the foreground or background grayscale, or color, to a specific grayscale value or index.

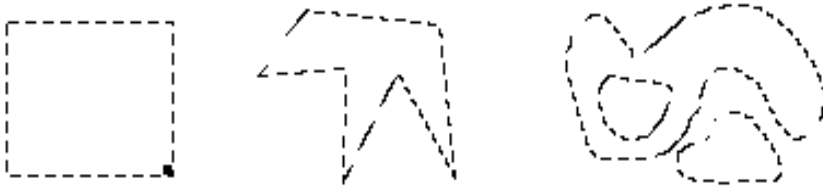
Gray Map Window - X and Y display the X-Y coordinates of the gray map control point you are currently changing by dragging the mouse. To change one of the two control points you click and drag near the border of the gray map.

Histogram Window - X is the current X-axis(grayscale) value, **Y** is the number of pixels in the selection which have that value.

Plot Window - X is the cursor x-coordinate. **Y** is the corresponding plot y value.

Using Selections

A selection is a user defined region of interest created using one or more of *Image's* five outlining tools. Selections are used for defining areas to be measured or areas to be operated on using various filtering or editing commands. Selections, such as those shown below, are indicated by the selection marquee, sometimes referred to as “marching ants”.



Moving a selection. Selections can be moved by clicking within the selection and dragging. The Results window displays the coordinates of the upper left corner of the selection(or the bounding rectangle for non-rectangular selections) as it is being moved. Notice that the cursor changes to an arrow when it is within the selection. If you want to move the *contents* of a selection, rather than the selection itself, first do a Copy(Command-C) and a Paste(Command-V), then click within the selection and drag. Use the shift key to constrain movement to horizontal or vertical. The arrow keys can be used to nudge the selection one pixel in any direction.

Stretching a Selection. Rectangular selections can be stretched using the handle in lower right corner. The contents of the selection will also be stretched if the selection is the result of a paste operation. The width and height will be displayed in the Results window as the selection is stretched.

Adding to a Selection. If you hold down the Control key(notice the little plus sign in the cursor) while making a selection, then the new selection you create will be added to the current selection. This feature allow you to edit existing selections, or to create discontinuous selections, such as the one above. This feature is implemented using QuickDraw's UnionRgn routine.

Subtracting from a Selection. If you hold down the Option key(notice the little minus sign) while making a selection, then the new selection you create will be subtracted from the current selection. This feature allow you to edit existing selections, or to create selections with holes in them, like the one above. This feature is implemented using QuickDraw's DiffRgn routine.

Deleting a Selection. To delete the current selection, choose any of the selection tools and click anywhere outside the selection. Or, alternately, choose a tool other than one of

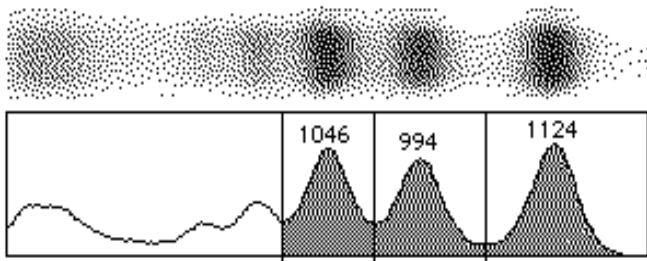
the selection tools, the magnifying glass, or the grabber hand. You can use Restore Selection to bring the selection back after you have deleted it.

Transferring a Selection. A selection can be transferred from one image window to another by using the Restore Selection command. Simple activate the window you want to transfer the selection to by clicking on it, and use Restore Selection. You can also use the Next Window command to activate another image window.

Saving a Selection. A selection can saved to disk using Save Outline As and restored later using the Open command.

Analyzing Electrophoretic Gels and Chromatograms

Here is one possible procedure for using *Image* to analyze a one dimensional electrophoretic gel(or a chromatogram), such as the one shown below. This procedure also demonstrates some of the less obvious features in *Image*, and also illustrates a few shortcuts.



- 1) Digitize the gel using a high quality monochrome TV camera and light box. Note that the gel must be oriented horizontally, not in the usual vertical mode. (You may want to first digitize a blank field so *Image* can do a shading correction). Save the digitized image.
- 2) Make space for the profile plots by selecting an area below the lanes of interest with the rectangle selection tool and pressing delete to erase it, or by opening a new Image window.
- 3) Use the Selection Rectangle tool to outline the first lane, *noting the width of the selection* shown in the Results window.
- 4) Double-click on the profile plot tool to bring up the Profile Plot Options Dialog box. Check Fixed Scale and Fixed Plot size, and uncheck Labels (Image will remember the settings if you use Record Preferences). Set the Plot Width to that of the selection width from step 3. You may also want to set Min and Max to more appropriate values. You can use the Measure or the Column Average Plot command to help determine optimum Min and Max values.
- 5) Use the Column Average Plot command to create the profile plot of a lane. Use Restore Selection if the selection outline you created in step 3 has disappeared.
- 6) Use Copy to copy the plot to the Clipboard. Click on the gel (or on the new) window and Paste. You may use the mouse to reposition the pasted plot, but *do not click outside of it prior to restoration of the original selection*, or the latter will be lost. Repeat steps 5 and 6 for each lane.
- 7) Double-click on the LUT tool to enable density slicing. Select the paint bucket tool, click in the LUT window within the density slice, and click to fill peaks in the profile

plots. You may first need to draw base lines, which must be black, with the ruler to create closed peaks. (Rectangular selections can usually serve as drop lines, or these may also be drawn with the ruler.)

8) Use Measure (Command-W) to find the area under peaks *within a selection outline*. In many cases, a single selection outline can be moved over each peak, otherwise draw a new one. (Measure will only count pixels within the outline that are highlighted by the density slice.)

9) Select the text tool and click on black in the LUT window.

10) Select 9-Point and Align Center from the Style menu.

11) Option-click with the text tool to automatically label the peaks, *in reverse order*, with the area measurements from step 8. The measurements will also have been recorded in tabular form, and can be printed (use Show Results to bring up the table).

Redirected Sampling

Here is a technique you can use to get Image to outline objects on one image, but get the pixel data used for density measurements from the corresponding area of another image.

- 1) Open(or digitize) two images. It helps if the two windows are placed side-by-side and are non-overlapping, but this is not necessary.
- 2) Outline a region of interest on one of the images.
- 3) Use Next Window(Command-`) to activate the other window.
- 4) Use Restore Selection(Command-4) to transfer the selection to the other window.
- 5) Use Measure(Command-1) to measure the corresponding region of interest in the second window..
- 6) Switch back to the original window using Next Window(Command-`).

If you are making many measurements you can use a macro utility, such as MacroMaker or QuickKeys, to automate steps 3-6. You might want to add a Number Selection command between steps 2 and 3 in order to outline and number the regions of interest for reference purposes. One, or both, of the windows can be magnified and this technique will still work. It will not work if more than two image windows are open.

Using the Option Key

Holding the option key down when using many of the commands and tools in *Image* causes alternative functions to be performed. You only need to hold the option key down when you first invoke a menu command.

Menu Command Options

Starting Image - Use QuickCapture card instead of Scion card, assuming both are installed

Open - Changes to Open All. (Hold down the command key to display images using current color palette instead of palette stored in file.)

Close - Changes to Close All

Print - Do bitmap printing(which is better for text and line art), rather than halftoning. (If using LaserWriter 6.0(or later) causes the driver(rather than *Image*) to do halftoning.)

Rotate Left, Rotate Right - Erase before rotating

Rotate and Scale - Use nearest neighbor sampling instead of bilinear interpolation

Smooth - More(unweighted) smoothing

Sharpen - Increased sharpening

Trace Edges - Result is not thresholded, allowing you to do it yourself

Convolve - Open kernel as an image

Calibrate - Allows you to edit the measured values

Start Digitizing - Digitize with Scion frame grabber using whole screen

Stop Digitizing - Omit shading correction

Average Frames - Sum frames instead of averaging

Run Movie - Erase screen before doing animation

Photo Mode -Move window to top of screen before erasing screen

Stack Windows - Move all windows to the “home” position in the upper left corner of the screen

Tile Windows - Use “Scale to Fit” mode to draw windows

Tool Options

Magnifying Glass - Zoom out instead of zooming in

Text Tool - Draw results of area or density measurements

Eraser - Pick up background color from image window

Brush - Pick up foreground color from image window

Ruler - Measure length of irregular path

Profile Plot Tool - Draw line

Eye dropper - Select background color rather than foreground color

Questions and Answers

Q. I am used to black having a value of zero, and white a value of 255, but in Image black is 255 and white is zero. How can I change this?

A. Choose one of the selection tools and use the Measure command twice (it doesn't matter what you measure). Hold down the option key and bring up the Calibrate dialog box. Enter 0 and 255 in the first column(replacing what was there) and 255 and 0 in the second column. Click the button labeled Straight Line and then click OK.

Q. How can I create a new window of a particular size, for example 1024x1024?

A. Bring up the More Options dialog box(its in the Options menu). Set New Height to 1024 and New Width to 1024 and click on OK. Any windows created with the New command during this session should now be 1024x1024. Many functions(such as Copy, Paste, and Undo) will fail with this large window unless you also make the Undo buffer larger. In the case of a 1024x1024 window, you would need to set the Undo buffer to 1024K.

Q. When I rotate a large rectangular object it gets clipped. How can I get around this problem?

A. Create a larger window(see question above), copy the object into the larger window, and then rotate it.

Q. Sometimes, after I have copied an image to the clipboard, the Paste command becomes dim. Why is this?

A. Images copied to the clipboard are stored in a buffer(called the Clipboard buffer), which is the same size as the Undo buffer. In some situations, such as filtering or 3D plotting, *Image* has to use the Clipboard buffer for internal operations. When this is necessary, the Paste command gets dimmed out.

Q. Why do the Finder's icons change color when I run *Image*?

A. *Image* all but two of the 256 available screen colors. The two "colors" that *Image* will never change are black and

white. Objects on the screen that are not black and white are likely to change color when in *Image* is being used. Colored objects are redrawn in their original color when you quit Image.

Q. Why do the colors get messed up when I drag an image window from one monitor to another?

A. *Image* does not support multiple monitors. The grayscale(or color) windows used by *Image* are only displayed correctly on the primary monitor, which is the monitor with the menu bar. The Control Panel can be used specify which monitor displays the menu bar.

Q. When I use Apple LUT, Make Binary, or 3D Plot my Mac IIcx hangs. Why does it do this?

A. You are probably processing an image that you have just acquired with the Data Translation QuickCapture card. There is a hardware bug with the QuickCapture card that causes this problem on some 68030 based Macs. Data Translation is working on a fix. You can temporarily work around the problem by Duplicating the Camera window and working with the copy.

Q. Sometimes when I use the Apply LUT, Make Binary, or 3D Plot command my Mac locks up and I have to restart. Why is this happening?

A. You probably are using a 68030 based Macintosh, such as the IICx, and have just captured an image using the Data Translation QuickCapture card. There is a hardware problem with the memory on the QuickCapture card that can cause the Mac IICx, and possibly other 68030 based Macs, to freeze up. You can work around the problem by Duplicating the Camera window before using one of the commands that cause the problem.

Q. Why don't the Halftone and BitMap buttons in the print dialog box do anything?

A. You are using version 6.0, or later, of the LaserWriter driver. This version supports halftone printing for all Mac applications. This feature is normally not used by *Image* since *Image* does its own halftoning by sending PostScript commands directly to the printer. You can disable *Image*'s built-in halftoning by holding the Option key down at the start of printing, in which case, you can take advantage of the buttons in the Print dialog box.

Using *Image* with Flatbed Scanners

Several potential problems can occur when you use a flatbed scanner to digitize images for use with *Image*. For example, scanners which only support 4-bit (16 gray level) scanning are a poor choice for use with *Image*, since at least 32 gray levels are need to realistic looking images, and 256 gray levels are desirable for quantitative analysis. You should also avoid scanners with software that is unable to save in uncompressed 8-bit TIFF format, since PICT files digitized at greater than 75 dots per inch(DPI) will probably not be displayed correctly by *Image*. The Apple scanner is an example of a scanner that does not work well with *Image*, since it is limited to 16 gray levels and is unable to save in grayscale TIFF format. An example of a scanner that does work reasonable well is the DataCopy 730GS.

You also have to be careful not to generate files that are too large for *Image* to handle. *Image* was optimized to handle the 640 x 480 (300K) images produced by frame grabber cards, whereas an 8 x 10 inch page scanned at 300 DPI by a flatbed scanner is 2400 x 3000 pixels , or 7.2MB, which is much larger than *Image* was designed to handle. The following table gives suggested scan areas for different scanning resolutions for use with the standard Apple 13 inch monitor and for 19 inch(1024 x 768) monitors. *Image* can

handle scans somewhat larger than these, but you will probable run into problems if you greatly exceed these recommended sizes.

	75 DPI	150 DPI	300 DPI
13" Monitor	7.5" x 6"	3.75" x 3"	1.8" x 1.5"
19" Monitor	12" x 10"	6" x 5"	3" x 2.5"

The Undo buffer should be set to 700K when working with scans the size of the ones suggested for 19 inch monitors.

You should probably stick to 75 DPI unless you are scanning small selections. 75 DPI also has the advantage of producing images that are near actual size when displayed on the screen, since nominal screen resolution is 72 DPI.

Appendix A: Frame Grabber Cards

Image supports two frame grabber cards for acquiring images from video sources, such as TV cameras and VCRs.

1) Data Translation QuickCapture

Part Number: DT2255-60Hz

Price: \$1295

Requires EP205 Cable Assembly: \$125

Data Translation, Inc.
100 Locke Drive
Marlboro, MA 01752
(508) 481-3700

2) Image Systems Technology(was Scion) Video Image 1000

Price: \$1195

Image Systems Technology
3 North Main Street
Walkersville, MD 21793
(301) 845-4045

Both cards have advantages and disadvantages. The DT2255 has a half megabyte of onboard memory, 300K of which *Image* uses for storing captured images. When using the DT2255, *Image* allows you to adjust brightness, contrast, and pseudocoloring while digitizing. The continuous histogram and live paste features are also currently only available with the DT2255. On the other hand, when capturing using the DT2255, the screen is redrawn only about four frames a second. Also, the A/D converter in the DT2255 seems to favor odd pixels values, which results in strange looking histograms. This does not, however, seem to be a problem in normal use, and Data Translation claims to have a fix.

The Scion card, in addition to being less expensive, has the amazing ability to capture and displaying on the screen at 30 frames per second. One disadvantage of this card is that, since it copies video data directly to the video card over the NuBus at video rates(about 9 MB/sec.), it doesn't work with some third party video cards, or is unable to capture the entire 640x480 frame. On the 640x480 Apple video card, it is able to capture 600x480

frames. The Scion card has a 4-8% vertical shading problem and is also considerably noisier than the QuickCapture card, problems which Image Systems claim they will fix.

Both Data Translation and Scion offer optional software for their respective cards for digitizing in color using an RGB color camera, and then reducing the resulting 24-bit color image to an 8-bit color PICT file that can be opened in *Image*.

Appendix B: Recommended Camera and Light Box

Quantitative image analysis using *Image* requires the use of a high quality TV camera and light box.

The Sierra Scientific MS-4030 solid state camera is one camera that is well suited for quantitative densitometry. Sierra Scientific also sells lenses and filters.

Imaging Research, creator of the highly regarded MCID biomedical image analysis system for the IBM-PC, makes a stabilized fluorescent illuminator that provides the uniform illumination needed for autoradiographic densitometry. They also sell several needed accessories, including the Nikon Micro-Nikkor f2.8/55 mm lens, the F-C adapter needed to connect the lens to a TV camera, and the Kaiser RS-1 copy stand. In addition, they will also sell the Sierra Scientific MS-4030 camera.

1) Sierra Scientific

1173 Borregas Avenue
Sunnyvale, CA 94089
408-745-1500

2) Imaging Research Inc.

Brock University
St. Catharines, Ontario
Canada L2S 3A1
416-688-2040

Appendix C: Alternate Mac II Analysis Systems

1) BigImage

2Ai Inc.
300 East Bells, #304
Pasadena, CA 91101
818-449-9512

2) The Explorer

UCLA Software Service Center
1400 PVUB
University of California, Los Angeles
405 Hilgard Ave.
Los Angeles, CA 90024
213-825-0757

3) Image Analyst

Automatix
1000 Tech Park Drive
Billerica, MA 01821
505-667-7900

4) NCSA Image

NCSA Documentation Orders
152 Computing Applications Building
605 East Springfield Avenue
Champaign, IL 61820
217-244-0072

5) NuVision

Perceptics Corporation
Pellissippi Center
P.O. Box 22991
Knoxville, TN 37933
615-966-9200

6) Prism Image Analysis System

Dapple Systems, Inc.
355 West Olive Ave.
Sunnyvale, CA 94086
408-733-3283

7) RIMS (Report and Image Management Systems)

MIPS - Medical Image Processing Specialists
6087 Jackson Rd.
Ann Arbor, MI 48103
313-655-5400

8) Ultimage

GTFS Inc.
2455 Bennett Valley Rd, #100C
Santa Rosa, CA 95404
707-579-1733

Appendix D: File Formats

TIFF Files Created by *Image*

Bytes 0-7 contain the TIFF header. Bytes 8-97 contain the TIFF directory. Within the TIFF directory, bytes 30-31 contain the picture width, and bytes 42-43 contain the picture height.

Bytes 98-255 are currently unused and are set to zero. Bytes 256-767 contain a 512 byte header which is unique to the *Image* program. This header is located at bytes 0-511 in the IPIC format. Image data starts at byte 768, stored in row order, one byte per pixel.

The information provided here should be adequate for writing a program to read a TIFF created by *Image*. It is not sufficient for creating TIFF files that *Image*, or any other program, can read. More detailed information on TIFF is available from either Aldus Corporation or Microsoft at the following addresses:

Developer's Desk
Aldus Corporation
411 First Ave. South
Suite 200
Seattle, WA 98104
(206) 628-6593

Windows Marketing Group
Microsoft Corp.
16011 N.E. 36th Wy.
P.O. Box 97017
Redmond, WA 98073

MCID File Format

The easiest way to create variable sized image files that can be read by *Image* is to duplicate the MCID format accepted by the Import command. These files contain the line width less one in bytes 0-1, the number of lines less one in bytes 2-3, followed by the 8-bit pixel data in row order. The 2 bytes (one 16-bit word) used to store the width and height are stored in Intel/VAX byte order, where the low order byte is first. The Import command expects these files to be of type TEXT, although they are actually binary files. The following Pascal program is an example of how to create a file that can be Imported into *Image*.

```
PROGRAM MakeRamp;  
{  
This example Pascal program that creates a picture  
file that can be opened using Image's Import command.  
}  
VAR
```

```

f:text;
i,line,pixel,PixelsPerLine,nLines:integer;
BEGIN
write('Pixels Per Line: '); readln(PixelsPerLine);
write('Number of Lines: '); readln(nlines);
writeln('Creating image. This may take a few minutes.');
```

```

rewrite(f,'Ramp');
write(f,chr((PixelsPerLine-1) MOD 256));
write(f,chr((PixelsPerLine-1) DIV 256));
write(f,chr((nlines-1) MOD 256));
write(f,chr((nlines-1) DIV 256));
FOR line:=1 TO nlines DO
  FOR pixel:=0 TO PixelsPerLine-1 DO write(f,chr(pixel));
close(f);
END.
```

IPIC Format

IPIC files, which *Image* can open but longer create, use a very simple file format consisting of a 512 byte header followed by uncompressed 8-bit pixel data. The format is similar to that used by the PDP-11 version of *Image*, and, in fact, the Macintosh version of *Image* can read the files produced by PDP-11 version of *Image*. The easiest way to generate a file that the Mac version of *Image* can read is to duplicate the PDP-11 file format, where bytes 0-1 contain the number of lines using Intel/VAX byte order and bytes 2-3 contain the number of pixels per line, also using Intel/VAX byte order. Image data starts at byte 512, and is stored in row order. Pixels are always one byte(8-bits) in length, where 0 is black(minimum intensity) and 255 is white(maximum intensity). The file type and creator of the file must be IPIC and IMAG, respectively.

Palette File Format

Pseudocolor palette files, which are of type ICOL, contain the number of colors in byte 0. Byte 1 contains the starting location of the palette within the LUT and byte 2 specifies how many times each color is replicated. Bytes 3-31 are currently unused, and are set to zero. Bytes 32-63 are used for storing red, bytes 64-95 for storing green, and bytes 96-127 for storing blue. In other words, starting at byte 32, 32 unsigned bytes are used for storing the red intensity values, then 32 bytes are used for storing green intensity values, and finally, 32 bytes are used for storing blue.

Appendix E: Program Limitations

The maximum number of pixels per line is 2048. There is no arbitrary limit (other than memory requirements) on the maximum number of lines in an image. *Image* uses two 300K buffers as Undo and Clipboard buffers. Many program functions will not work with images larger than these buffers. The size of these buffers can be changed in the More Options dialog box. You must Save Preferences, Quit, and then restart *Image* before the buffer size change will take effect.

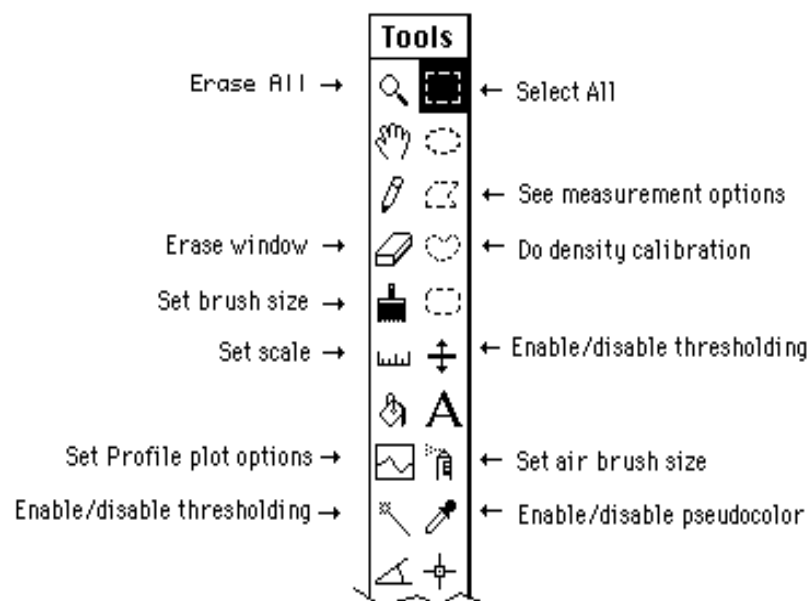
The maximum number of colors allowed in a pseudocolor palette is 32. This will probably be increased in some future version.

The maximum number of characters that can be saved, printed, or copied to the clipboard is 10,000.

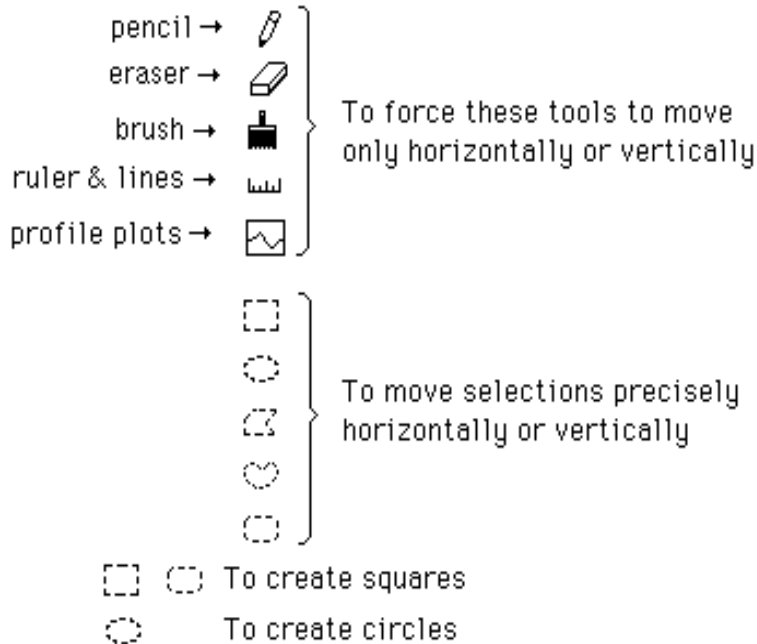
The maximum number of area (density) measurements is 200. The maximum number of length measurements is 100 and the maximum number of X-Y coordinate pairs that can be recorded is 500. The maximum number of allowed density standards is 20.

Appendix F: Shortcuts

Double-Click on tool to:



To constrain tool functions hold down the shift key:



Appendix G: Updated Versions and Bug Reports

Image is available from the National Technical Information Service(NTIS), and from many Macintosh bulletin boards and user group libraries. A reasonably current version, including Pascal source code and example images, should be available from one of the following:

- 1) From a friend. The *Image* program, including source code and documentation, is public domain and may be freely copied, distributed, and modified.
- 2)
DL10 of MACPRO on CompuServe (expensive)
- 3)
Twilight Clone BBS in Silver Spring, MD, 301-946-5032 (\$25/year fee)
- 4)
Via anonymous FTP from alw.nih.gov [128.231.128.251] in the directory /pub/ftp/image

- 5)
Via anonymous FTP from sumex-aim.stanford.edu [36.44.0.6]
- 6)
EMMPDL(Electron Microscopy and Microanalysis Public Domain Library),
312-972-7919, Username: EMMPDL, Password: EMMPDL, 1200 baud, 8 data, 1
stop,
No parity (user pays phone charges)
- 7)
NTIS, 703-487-4650, Order Number PB89-212732, \$100(Check, Visa,
MasterCard).
NTIS sells a two disk package complete with documentation, HyperCard reference
stack,
Lightspeed Pascal source, and example images.

Bug reports and suggestions for future versions are welcome. The author can be reached
at any of the following electronic mail addresses:

BitNet:	wsr@nihcu
Internet:	wayne@alw.nih.gov
CompuServe:	76067,3454